How does defoliation management impact on yield, canopy forming processes and light interception of lucerne (*Medicago sativa* L.) crops?

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Abstract

The frequency of defoliation is the major management tool that modulates shoot yield and the accumulation of C and N root reserves in lucerne crops. A fully irrigated, two-year old lucerne (*Medicago sativa* L.) crop was grown at Lincoln University (43°38'S and 172°28'E) and subjected to four defoliation treatments. These involved the combination of two grazing frequencies (28 or 42 days) applied before and/or after mid-summer. Annual shoot dry matter (DM) yield ranged from 12 to 23 t/ha. These differences were largely explained by the amount of intercepted photosynthetically active radiation (PAR_i) using a conservative conversion efficiency of 1.6 g DM/MJ PAR_i. Part of the reduced PAR_i in the frequently defoliated treatments was caused by the shorter regrowth period that impeded crop canopy closure to the critical leaf area index (LAI_{crit})

of 3.6. Canopy architecture was unaffected by treatments and a single extinction coefficient for diffuse PAR_i (k_d) of 0.81 was found for 'Grasslands Kaituna' lucerne. The pool of endogenous nitrogen (N) in taproots was reduced by frequent defoliations. This explained differences in leaf area expansion rate (LAER) which decreased from 0.016 $m^2/m^2/ºCd$ at 60 kg N/ha to 0.011 $m^2/m^2/ºCd$ at 20 kg N/ha. The pool of soluble sugars was also positively associated with LAER but the concentrations of carbohydrates and N reserves and the pool of taproot starch were poorly related to LAER. The slower LAER in the frequently defoliated treatments was mostly caused by the smaller area of primary and axillary leaves, particularly above the 6^{th} node position on the main-stem. On the other hand, developmental processes were less affected by defoliation frequency. For example, the phyllochron was similar in all treatments at 34ºCd per primary leaf during spring/summer but increased in autumn and ranged between 44 and 60ºCd. Branching started after the 4^{th} main-stem node and leaf senescence after the 3^{rd} main-stem node, but both were unaffected by defoliation frequency. These results suggest that the expansion of individual leaves, both primary and axillary, was the most plastic component of canopy formation, particularly after the appearance of the 6^{th} primary leaf. Future mechanistic modelling of lucerne crops may incorporate the management or environmental responses of LAER that control PAR_i and impact on shoot DM yields.

Key words: alfalfa, branching, grazing management, leaf area index, phyllochron, root reserves, senescence.

Introduction

The shoot yield of lucerne (*Medicago sativa* L.) is linearly related to the amount of solar radiation intercepted by the crop canopy during each regrowth cycle (Gosse *et al.*, 1984). This is consistent with lucerne best management practices (BMPs) that advise rotational grazing to maximize rates of canopy expansion and enhance light capture and yield (Moot *et al.*, 2003). The frequency and timing of defoliation is therefore a critical management decision that influences yield, nutritive value and persistence of lucerne stands (Belanger *et al.*, 1999; Keoghan, 1982). Historically, it is well known that, when lucerne crops are frequently defoliated there is a significant reduction in shoot growth rates and limited accumulation of carbon (C) and nitrogen (N) reserves in crowns and taproots (Graber *et al.*, 1927; Reynolds and Smith, 1962). Low levels of endogenous reserves reduce yield in subsequent regrowth cycles because they are readily mobilized to support shoot regrowth, particularly after defoliation and during early-spring (Louahlia *et al.*, 1998; Teixeira *et al.*, 2007b). However, it is unclear how frequent defoliation, and the consequent low levels of endogenous reserves, affects the canopy forming processes and subsequent light interception in lucerne crops. This understanding is crucial to improve lucerne BMPs and enhance the accuracy of lucerne simulation models (Confalonieri and Bechini, 2004; Fick *et al.*, 1988).

The accumulated photosynthetically active radiation intercepted during a production cycle ($\sum PAR_i$) depends on the (i) availability of above-canopy incident PAR (PAR_o), (ii) canopy cover (i.e. leaf area index, LAI) and (iii) canopy architecture (Monteith, 1994). Under controlled conditions, Justes *et al.* (2002) reported that lucerne crops with reduced

levels of endogenous reserves had lower LAI expansion rates but the mode of action was not reported. A reduction in LAI expansion could be caused through (i) lower rates of organ development (i.e. slower differentiation of meristems into shoots, leaves or branches); (ii) limitations to growth processes (e.g. leaf expansion), (iii) faster rates of tissue senescence, and/or (iv) changes in canopy architecture that reduce interception of PAR per unit of LAI. Additionally, canopy forming processes in lucerne can be seasonally influenced by photoperiod and temperature (Brown *et al.*, 2005). Temperature strongly modulates the rates of plant development which are usually conservative when expressed on a thermal-time (degree-day, ºCd) basis (Bonhomme, 2000). Nevertheless, in lucerne, leaf area expansion rate (LAER, $m^2/m^2/ºCd$) is faster in spring/summer than in autumn (Gosse *et al.*, 1984; Gosse *et al.*, 1982) when the phyllochron (i.e. thermal units required for the appearance of a leaf) is reduced (Brown *et al.*, 2005). This seasonal response of the canopy formation components has not been tested in field grown crops subjected to frequent defoliations or with low levels of endogenous reserves. The aim of this research was to quantify seasonal LAI and describe the underlying processes of canopy formation in lucerne crops subjected to contrasting defoliation regimes (frequencies and timings) that created different shoot yields and levels of endogenous reserves (Teixeira *et al.*, 2007b).

Materials and Methods

Experimental site and defoliation treatments

From 14 June 2002 to 04 October 2004, four defoliation treatments were applied on an established two year old crop of 'Grassland Kaituna' lucerne in Canterbury, New Zealand (43°38'S, 172°28'E, 11 m a.s.l). Treatments were distributed in a completely randomized block design (4 replications) as a combination of (i) two grazing cycles (28 or 42 days) and (ii) two timings of application of grazing cycles: the first and/or second half of the growth season (Table 1). Specifically, for two treatments a constant grazing cycle of 42 days (L, long cycle) or 28 days (S, short cycle) was applied throughout the year (LL and SS crops, respectively). For LS and SL crops, the 42 and 28-day grazing cycle were applied from early-spring to mid-summer (4 February) respectively, and then switched to the alternative grazing cycle for the remainder of the year. The aim of the switch treatments was to uncouple management and seasonal effects and create crops with intermediate levels of root endogenous reserves by alternating the grazing cycle during mid-summer/autumn, when crops preferentially accumulate reserves in crowns and taproots (Cunningham and Volenec, 1998; Teixeira *et al.*, 2007b).

[Table 1 suggested place]

Crops were grazed by sheep of mixed age classes and any post-grazing stem residual was trimmed to a height of ~50 mm to aid measurement of new shoot regrowth but avoid damage to the crown or emerging basal shoots. The soil in the experimental area is a Wakanui deep silt loam (*Aquic Ustochrept*, USDA). Crops were irrigated and fertilized for optimal yields and weed invasion was minimized by chemical control. Additional

details about the site, climate, fertilization and crop management were given in Teixeira *et al.* (2007b).

Measurements

Air temperature and solar radiation

During the 2002/03 growth season, global solar radiation (R_o, MJ/m^2) was monitored using a pyranometer LI-200SA (LI-COR Inc., Lincoln, Nebrasca, USA) and the air temperature (T_{air}, °C) was measured by a thermistor installed at ~1.50 m above ground. Both R_o and T_{air} were measured every minute and the average recorded at each 1 hour interval by a CR-10 datalogger (Campbell Scientific, Logan, Utah, USA). The thermistor sensor was installed inside a 0.2 m long polished aluminium tube shelter to remain protected from direct solar radiation. It was calibrated to a mercury thermometer with 0.1ºC precision.

During the 2003/04 growth season, a DataTaker logger model DT600 (dataTaker Australia Pty Ltd 7, Victoria, Australia) was used. In addition, three tube solarimeters model TSM (Delta-T Devices, Cambridge, UK) were installed at ground level in two plots of the LL treatment. The measurements of solar radiation above the canopy were taken by the same pyranometer used in the first season (LI-200SA) installed 1.8 m above ground. In 2003/04 measurements were taken at 30 second intervals and then averaged

and logged at hourly intervals. Phothosynthetically active radiation (PAR) was assumed as half of the global incoming solar radiation (Szeicz, 1974).

Sampling of shoot and taproot dry matter (DM)

Shoot DM samples were taken each 7-10 days within grazing cycles starting about 10 days after the previous grazing. The last sampling of each cycle was taken 18-24 h prior to the entrance of the grazing animals in the paddock. Shoots samples were cut above crown level from the area of a single 0.2 m^2 quadrat placed randomly in each plot. The material was dried in a forced air draft oven at 65°C for at least 48 hours to a constant weight.

Taproot biomass (kg DM/ha) was used as an indicator of the differences in the pools of endogenous reserves (Teixeira *et al.*, 2007b). Samples of taproot were collected from a depth of 300 mm in the same 0.2 m^2 area where shoots were harvested. Taproots were washed free of soil under a stream of cold water, freeze dried, weighed and stored for further chemical analysis. Taproot samples were analysed for the concentration of carbohydrates (soluble sugars and starch) and nitrogen in the dry matter. The laboratory methods are given in detail in Teixeira *et al.* (2007b). In brief, total nitrogen was analysed from 500 mg samples of taproot dry tissue using the Kjeldahl protocol (AOAC, 1995). Soluble sugars were extracted from taproots by repeatedly incubating freeze-dried samples in ethanol 80%. Three sub-samples of 10 µl were transferred to the wells of a microplate and 2 µl of phenol and 100 µl of H_2SO_4 were added to each well. The

remaining ethanol-insoluble residue was imbibed in 1 ml 2N KOH to gelatinise the starch. After pH adjustment (1 ml of 2N acetic acid), starch was digested with ~50 U of amyloglucosidase (Sigma Chemical, St. Louis) in a water bath at 45°C for 1 h. Samples were then centrifuged at 3500 rpm for 4 minutes. Microplate wells were filled with 20 µl of samples (3 subsamples/sample). In each well 100 µl of glucose hexokinase (Sigma Chemical, St. Louis) was added. Absorbances were read in a microplate reader Model Ceres 900 (Bio-Tek Instruments Inc., Winooski, VT, USA) at 400 nm wavelength for soluble sugars and 340 nm for starch. Readings were compared with a calibration curve based on sucrose and glucose standards.

Total pools of carbohydrates and nitrogen were calculated as the product of the concentration of reserves (% of taproot DM) and total taproot DM (kg DM/ha).

Count of green primary leaves and senesced primary leaves

Fully expanded primary leaves were counted from 80 marked shoots (5 per plot) on 255 dates from 22 August 2003 to 8 June 2004. At the beginning of each regrowth cycle a group of five "Dominant-shoots", i.e. shoots within the group of the 33% tallest shoots (Teixeira *et al.*, 2007a), from different plants were marked in each treatment plot. Starting from 7-10 days after grazing, these marked shoots were assessed every 5-7 days for the number of fully expanded primary leaves during both years and, in 2003/04, also for the number of senesced leaves. A leaf was considered senesced when more than half of its area was pale-yellow or when the leaf had detached from the node. Marked shoots that

exhibited an early senescence or death within the regrowth cycle were discarded and replaced with new Dominant-shoots. This bias was intentionally used to ensure that only Dominant-shoots were measured because they contributed to the majority of the above ground yield (Teixeira *et al.*, 2007a).

Phyllochron calculation and branching measurements

To calculate the phyllochron, the number of primary leaves per shoot was plotted against thermal-time accumulation of each regrowth cycle. Thermal-time (Tt, ºCd) was calculated using a broken-stick framework with a base temperature (T_b) of 5ºC, an optimal temperature (T_{opt}) of 30ºC and a maximum temperature (T_{max}) of 40ºC (Fick *et al.*, 1988). This simple thermal-time calculation framework was sufficiently accurate for quantifying leaf appearance rates in the present experiment (Teixeira, 2006) despite more sophisticated models, as proposed by Brown *et al.* (2005) for Canterbury conditions. The slope of the linear regression between the number of primary leaves and accumulated Tt (∑Tt) represents the phyllochron (°Cd/leaf). In addition, the hypothesis of a seasonal pattern of change in lucerne phyllochron (Brown *et al.*, 2005) was tested by plotting it against the average photoperiod (Pp) of each regrowth cycle.

The number of axillary leaves at each main-stem node (i.e. branching) was counted during the summer-autumn period of 2003/04 (19 February 2004 to 5 May 2004). This occurred on the same dates and on the same marked shoots used for the counting of primary leaves.

Individual area of primary and axillary leaves

Leaf area of individual primary and axillary leaves was measured on three occasions from 1 October 2003 to 16 March 2004. For each sampling date, 20 Dominant-shoots (5 per plot) of LL and SS treatments (i.e. treatments with consistent defoliation frequencies through the year) were harvested. Leaves of each main-stem node position were detached, opened flat on a white sheet of A4 paper and digitally photographed with a Nikon camera model CoolPix 950 (Nikon Co., Japan). Individual leaf area was measured using the image analyses software 'QUANT' (Vale *et al.*, 2003) and calculated by comparing the count of pixels in the leaf image with the one of a known reference line of 20 mm on the same A4 paper.

Accumulated PAR interception

Accumulated intercepted PAR ($\sum PAR_i$) was calculated by summing daily estimates of intercepted PAR (PAR_i) from each regrowth period. Daily PAR_i was obtained by multiplying the available above canopy PAR of each day (PAR_o) by the fractional PAR interception (PAR_i/PAR_o) for each treatment plot. Daily PAR_i/PAR_o was estimated from measurements of diffuse non-interceptance (DIFN) taken with a canopy analyser LAI-2000 (LI-COR Inc., Lincoln, Nebraska, USA). Readings of DIFN were taken in predominantly diffuse light conditions at 7 day intervals, starting 10 days after the last grazing day of the previous regrowth cycle. The equipment was set to take one reading

above and five readings below canopy in each plot. The canopy analyser LAI-2000 estimates the fractional transmission of diffuse light (i.e. DIFN) through the canopy at wave lengths lower than 490 nm from readings at five different zenith angles (7, 23, 38, 53 and 68°). Therefore, DIFN quantifies the fraction of sky that is not blocked by foliage (Jonckheere *et al.*, 2004) and represents $[1–(PAR_i/PAR_o)]$.

To evaluate the accuracy of 1-DIFN (diffuse PAR interception) as a measure of PAR_i/PAR_o, the relationship between 1–DIFN and the fractional solar radiation interception (R_i/R_o) was tested. This was done by regressing 1–DIFN values against daily R_i/R_o calculated from tube solarimeters data (Teixeira, 2006). The strong linear relationship (R^2=0.96) with a slope ($P<0.01$) of 1.03 ± 0.09 (95% confidence interval) and an intercept ($P<0.01$) of 0.08 (±0.06), indicated that the canopy analyser consistently measured 0.08 more fractional interception than the tube solarimeters regardless of the amount of canopy cover (Teixeira, 2006). This positive intercept would be expected because the canopy analyser measures wavelengths in the blue region of the solar spectrum (490 nm) while the tube solarimeters are sensitive to a larger band of the solar spectrum (400-2200 nm). Therefore, for this experiment, 1–DIFN was assumed to correctly represent fractional PAR interception (PAR_i/PAR_o) and daily values of PAR_i/PAR_o were estimated by linearly interpolating the measurements of 1–DIFN.

Leaf area index

Leaf area index (LAI) was estimated from calibrated computations of plant area index (PAI) taken with the canopy analyser simultaneously with DIFN readings. All readings at PAI<1.5 were taken with the sensor lens at ground level. This was done by placing the canopy analyser sensor inside channels (30 mm depth x 300 mm length) installed before the first measurement of each regrowth cycle. This procedure guaranteed that the transmittance of light by short canopies was measured by the sensors. When PAI>1.5 the readings were taken at sensor height (~30 mm above ground level).

To compensate for the bias expected when measuring clumped canopies (Nouvellon *et al.*, 2000), mainly in the initial stages of regrowth, PAI computations were calibrated with 110 destructive measurements of LAI taken from 2 June 2003 to 16 March 2004. The LAI increased linearly ($P<0.01$) with PAI with a slope of 0.93 (±0.05) and an intercept not different ($P=0.32$) from zero (Teixeira, 2006). Therefore PAI overestimated actual LAI by 7% and this was corrected by adjusting PAI (Equation 1).

$$LAI = 0.93 \times PAI \quad \text{(Equation 1)}$$

Destructive LAI measurements

During the first half of 2003/04 growth season, sub-samples of ~10 shoots were separated from the bulk of the shoot sample. All the leaves of the selected shoots were removed, opened onto a flat A4 white paper background and digitally photographed. Pictures were analysed with the image software 'QUANT' v.1.0.1 (Vale et al., 2003) and leaf area

calculated by comparing the number of pixels contained in the image of leaves with the ones in a 200 mm reference scale. Leaf samples were then dried in a forced air oven for at least 48 hours at 65°C to a constant weight. The average canopy specific leaf weight (SLW, g/m^2) was calculated as leaf mass per unit area of green leaf. Destructive leaf area index (LAI_{dest}) was then calculated as:

$$LAI_{dest} = DM_{leaf}/SLW \quad \text{(Equation 2)}$$

Where DM_{leaf} is the total amount of leaf DM (g/m^2) estimated for a given plot.

Extinction coefficient for diffuse radiation

The extinction coefficient for diffuse radiation (k_d) was used as an indicator of canopy architecture. The k_d was calculated as the linear slope between the natural log of diffuse PAR transmission [$1–(PAR_i/PAR_o)$] and LAI (Hay and Walker, 1989). For calculation purposes, the value of k_d for each treatment was estimated by plotting the natural log of DIFN (taken with the canopy analyser) with 110 independent destructive measurements of LAI (Equation 3) for the same respective sampling dates and plots between 2 June 2003 and 16 March 2004.

$$k_d = -\frac{\log\left[1-\left(PAR_i/PAR_o\right)\right]}{LAI_{dest}} \quad \text{(Equation 3)}$$

Data analyses

Linear and non-linear regressions were fitted between dependent and explanatory variables using SIGMAPLOT version 8.02 (SPSS Inc.). Where appropriate, to simplify non-linear patterns for simulation modelling purposes, bi-linear (broken-stick) functions were fitted by the use of a dummy variable to identify the inflection point of regressions which minimized the R^2 of the relationship (Draper and Smith, 1998). The differences in individual leaf area with node position were described by a bell-shaped function (Dwyer and Stewart, 1986) commonly used to model leaf area in cereals (Elings, 2000) (Equation 4).

$$Y = Y_0 \times \exp[a \times (X - X_0)^2 + b \times (X - X_0)^3] \quad \text{Equation 4}$$

Where Y is the individual leaf area (mm^2) at node position X, Y_0 is the mature leaf area of the largest leaf (mm^2), X is the leaf number counted from the base of the main-stem, X_0 is the position of the largest leaf in the main-stem, and *a* and *b* are empirical constants (Dwyer and Stewart, 1986). Parameter *a* quantifies the kurtosis or "breadth" of the curve whereby low values of *a* result in a sharp increase or decrease of the curve. Parameter *b* quantifies the degree of "skewness" of the curve with positive values resulting in curves skewed to the right (towards leaf positions greater than X_0).

The regression coefficients of equations and the results for each variable were tested using analysis of variance (ANOVA). In all cases, means were compared whenever treatment effects in the ANOVA presented $P<0.05$. Then, a Fisher's protected least

significant difference (LSD) was used to separate means at the 5% level (α=0.05). The software used for statistical analysis was GENSTAT 7th edition (Lawes Agricultural Trust, IACR, Rothamsted, UK).

Results

Annual shoot yield and accumulated PAR_i

Annual shoot yield ranged from ~1200 to 2300 g/m^2 (i.e. 12 to 23 t DM/ha/year) and was linearly related (R^2=0.90) to the amount of intercepted PAR (Figure 1). The relationship had a slope of 1.6 g DM/MJ PAR_i ($P<0.01$) and the intercept was not significantly different from zero (α=0.05).

[Figure 1 suggested place]

The extinction coefficient for diffuse light

Fractional PAR interception increased exponentially (R^2=0.92) with leaf area index and followed a similar pattern (P=0.95) for all treatments (Figure 2). The slope of the regression gave an extinction coefficient for diffuse PAR (k_d) of 0.81. Therefore, the critical leaf area index (LAI_{crit}), where 95% of the available PAR was intercepted, was 3.6 in all treatments.

[Figure 2 suggested place]

Seasonal leaf area index

The leaf area index (LAI) was frequently higher than the LAI_{crit} of 3.6 when crops were defoliated at 42 days intervals. A maximum LAI of ~6.0 was measured in LL treatments during summer (Figure 3 a). In contrast, in the frequently defoliated treatments, canopy cover was $<LAI_{crit}$ in most regrowth cycles. Specifically, LAI_{crit} was surpassed in ~77% of the regrowth cycles in the LL treatment (Figure 3 a), but only in 3 of the 18 regrowth cycles (17%) for SS treatment, namely Cycle 4 in 2002/03 and Cycle 5 during both growth seasons (Figure 3 d). LS and SL treatments had intermediary levels of LAI according to the time of the year (Figure 3 b and 3 c).

[Figure 3, suggested place]

Leaf area expansion rates (LAER)

The rate of LAI expansion per thermal-unit (LAER, $m^2/m^2/ºCd$) was reduced ($P<0.05$) by up to 20% in treatments previously subjected to frequent defoliations but the level of response differed depending on the regrowth cycle (data not shown). For example, differences were particularly evident for LS and SL treatments during the regrowth cycles immediately after the defoliation frequency switch. During this time, LAER was 0.127

$m^2/m^2/ºCd$ in LL but ~16% lower ($P<0.05$) in the SL treatment (0.107 $m^2/m^2/ºCd$). Similarly, for the short regrowth cycle, LAER was 0.118 $m^2/m^2/ºCd$ in LS but declined ($P<0.05$) to 0.096 $m^2/m^2/ºCd$ in the SS treatment.

The relationship between early-spring LAER and concentrations (% of taproot DM) or taproot pools (kg/ha) of winter reserves was estimated by linear regression (Figure 4). The LAER increased ($P<0.02$) with the total pool of taproot nitrogen ($R^2=0.76$) and total sugars ($R^2=0.72$) rather than the concentration of carbohydrates or nitrogen in taproots, or the pool of starch ($R^2=0.39$).

[Figure 4, suggested place]

The seasonal phyllochron

During the spring-summer period, the phyllochron (ºCd/primary leaf) was similar ($P=0.88$) among treatments at 34°Cd/leaf. It increased to 40-65°Cd during autumn-winter when the LL treatment had a shorter ($P<0.02$) phyllochron (~40°Cd/leaf) than LS (63°Cd/leaf) and SS (56°Cd/leaf) but was similar to the SL treatment (47°Cd/leaf). This seasonality caused the phyllochron to decrease exponentially ($R^2=0.76$) from 42°Cd/leaf at a photoperiod of 10.5 h to 34°Cd/leaf at 16.5 h in LL treatment (Figure 5 a). The critical Pp (Pp_{crit}), assumed as the Pp when phyllochron was 5% greater than the asymptote (Hodges, 1991), was 12.5 h for LL treatments. Therefore, at Pp greater than the Pp_{crit}, the phyllochron was similar among all treatments at 34°Cd/leaf. In contrast, for

photoperiods less than Pp_{crit}, treatments defoliated at short intervals post 4 February (LS and SS) had an 18% longer ($P<0.02$) phyllochron (51°Cd/leaf) than LL and SL treatments (43°Cd/leaf) (Figure 5 b and 5 c).

[Figure 5, suggested place]

Axillary leaf appearance (branching)

Branching, quantified as the total number of leaves (i.e. primary plus axillary) in relation to the number of primary leaves, followed a similar pattern ($P=0.57$) for all treatments (Figure 6). The appearance of axillary leaves started after the full expansion of the 4th primary leaf and progressed exponentially ($P<0.01$) at a similar rate ($P=0.31$) in all treatments. A bi-linear relationship ($R^2=0.98$; insert in Figure 6) indicated that axillary leaves appeared at an average rate of 3.1 leaves/primary leaf, until the expansion of the 9th primary leaf. After that, branching rate increased to 6.8 leaves/primary leaf until the expansion of the 11th primary leaf (the maximum number of primary leaves measured at that period).

[Figure 6, suggested place]

Senescence of primary leaves

Leaf senescence occurred at a similar (P=0.17) rate for all defoliation treatments. A broken-stick model explained 89% of the variation of the relationship between the accumulated number of senesced primary leaves and the number of main-stem nodes (Figure 7). The model indicated that senescence of the first primary leaf commenced at the time of appearance of the 3rd main stem-node. In this first stage, senescence proceeded at a rate of 0.2 primary leaves/main-stem node until the appearance of the 6th main-stem node when the rate increased (P<0.05) to 0.48 leaves/main-stem node.

[Figure 7, suggested place]

Area of individual leaves

Area of primary leaves

The area of each individual primary leaf (mm^2/leaf) increased from the smallest leaf at node position 1 (~120 mm^2) to a maximum at node position 8 (1000 to 2000 mm^2) in all treatments (Figure 8). At higher node positions leaf area decreased to 800-1000 mm^2 at node position 11 in LL treatments, which was the highest node position on which leaf area was measured.

From node position 1 to 6 there was no effect of defoliation treatments on primary leaf area. In contrast, from node positions 7 to 10, primary leaves in the SS treatment were 60-85% smaller (P<0.05) than in LL treatment.

Primary leaves of each node position were largest ($P<0.05$) in the regrowth of 16 March 2004 (mean of 1,110 mm^2) followed by 24 December 2003 (755 mm^2) and 1 October 2003 (455 mm^2) in both treatments (Figure 8).

[Figure 8, suggested place]

Area of individual axillary leaves

The total area of axillary leaves for each node position was affected by defoliation treatments and differed among seasons (Figure 9).

Axillary leaves were larger ($P<0.05$) in LL treatment than SS treatment from the 3rd to the 7th node position with no difference at higher or lower nodes. The greatest difference ($P<0.03$) was in the area of the largest axillary leaf (Y_0) that was on average 1,530 mm^2 for LL crops but 726 mm^2 on SS treatment.

There was also a seasonal effect on axillary leaf area in both treatments. In early-spring (1 October 2003) the area of individual axillary leaves from nodes 4 to 8 was smaller ($P<0.05$) than in early-summer (24 December 2003) or late-summer (16 March 2004).

[Figure 9, suggested place]

Discussion

PAR_i was affected by defoliation regimes

Accumulated PAR interception ($\sum PAR_i$) explained the majority of the differences in annual shoot yield among treatments (Figure 1). Therefore, the lower shoot yield observed in frequently defoliated treatments, which also had the lowest levels of endogenous reserves (e.g. Figure 4), was mainly attributed to the reduction in the amount of energy captured for photosynthesis. Nevertheless, lucerne yield is also dependant on the conversion efficiency of PAR_i into crop biomass and on the partitioning of DM between shoots and roots (Khaiti and Lemaire, 1992). Although the overall estimate of the radiation use efficiency (RUE) for "annual shoot DM" production was similar among treatments at 1.6 g DM/MJ PAR (Figure 1), there were seasonal and treatment differences in RUE for shoots and total biomass during individual regrowth cycles (Teixeira, 2006).

To understand the processes that caused the differences in the amount of intercepted PAR among defoliation treatments, individual components of canopy formation were then examined.

Canopy architecture was unaffected by defoliation regime.

The extinction coefficient for diffuse PAR (k_d) was used as an indicator of morphological changes in canopy architecture (Figure 2). The high value of 0.81 for k_d in 'Grasslands Kaituna' was consistent with previous reports for other lucerne cultivars such as 0.88 in

'du Puits'(Gosse *et al.*, 1988), 0.86 in 'Dekalb 167' (Whitfield *et al.*, 1986) and 0.91 in 'Aurora' (Evans, 1993). Furthermore, the consistency of k_d among seasons indicates the canopy architecture (i.e. mean canopy leaf angle) and leaf optical properties (e.g. light reflection) were unaffected by environmental signals, defoliation frequency or the level of endogenous reserves. This high and stable k_d suggests that the scope for morphological adjustments in response to frequent defoliations was limited. In fact, lucerne canopy architecture, with inclined leaves at the top and more horizontal leaves at the bottom, is already considered efficient for light capture (Duncan, 1971; Travis and Reed, 1983).

Treatment effects on canopy expansion

The lower interception of PAR observed in frequently defoliated crops (Figure 2) was caused by two factors. Firstly, the short regrowth length of 28 days limited $\sum PAR_i$ because the harvests mostly occurred before full canopy closure at LAI_{crit} (Figure 3 d). Secondly, canopy expansion rates (i.e. LAER) were reduced in frequently defoliated treatments (Figure 4) which also reduced the mean LAI (Figure 2) and hence the lower $\sum PAR_i$. Our data suggests that the lower availability of endogenous reserves in roots, particularly nitrogen, in the SS treatment was the cause of smaller leaves and consequent slower LAER in these crops (Figure 4). Leaf growth is strongly dependent on the availability of nitrogen for cell division and expansion (Simon et al., 2004; Thornton and Millard, 1997). Meuriot *et al.* (2005) observed that limited supply of taproot nitrogen to shoots, during early lucerne regrowth, reduced LAI development by delaying recovery of photosynthetic capability and nitrogen uptake. In the current experiment, LAER was only

moderately related (R^2=0.58) to the concentration of N in taproot DM but was strongly (R^2=0.76) associated with the amounts of taproot N (N pool in kg N/ha). This demonstrates that changes in the structural biomass of taproots, that was also reduced by frequently defoliations (Teixeira *et al.*, 2007b), was important in defining the storage capacity of endogenous N. In fact, studies suggest that canopy expansion may be more sensitive to specific N fractions of lucerne roots. Soluble proteins and vegetative storage proteins (VSPs) were more strongly associated to shoot growth rates than the total N content of lucerne roots (Avice *et al.*, 1997). These N fractions are highly mobilized from taproot reserves and translocated to shoots after defoliation and during early-spring (Volenec *et al.*, 1996). Justes *et al.*(2002) showed that both the concentration of soluble proteins and VSPs in lucerne roots were closely associated to LAER. In contrast, carbohydrate concentrations and starch pools did not show such strong relation with LAER (Figure 4 b, 4 c and 4 e). This is consistent with the idea that carbon root reserves are mostly respired instead of translocated to shoots (Avice *et al.*, 1996a). In addition, canopy expansion was shown not to respond instantly to carbon supply because there is a plasticity of leaf thickness, i.e. changes in specific leaf weight (Tardieu *et al.*, 1999). This experiment showed a positive association between LAER and the pool of soluble sugars. However, this relationship was caused by the changes in total root biomass because the long-term concentration of soluble sugars in roots was unaffected by defoliation treatments (Teixeira *et al.*, 2007b).

Interestingly, the differences in LAER were mainly caused by changes in the area of individual leaves particularly after the 6th main-stem node (Figures 8 and 9). The

insensitivity of the first expanded leaves to the level of endogenous reserves indicates that the supply of assimilates from roots to shoots was sufficient for full leaf expansion, even in the frequently defoliated treatments. These leaves could have been formed before the period of intense depletion of root reserves that occurs ~ 10 days post-defoliation (Avice *et al.*, 1996b). In the early stages of leaf growth, cell division is highly sensitive to nitrogen supply (Gastal and Nelson, 1994), and for the first expanded leaves this period probably have occurred close to the day of defoliation when N reserves are maximal. On the other hand, at later stages of regrowth, leaf expansion was reduced by frequent defoliations. The area of the largest leaf was reduced on average by 30% in primary and 50% in axillary nodes of the SS treatment when compared with the LL treatment (Figure 8). The area of the largest leaf was reduced on averaged by 30% in primary and 50% in axillary nodes of SS treatment when compared to LL treatment (Figure 8). After defoliation, expanding leaves become the major sink for taproot N reserves (Kim *et al.*, 1991) because exogenous sources of N (mineral uptake and atmospheric fixation) are limited (Kim *et al.*, 1993). The exact mode of reduction in individual leaf area during more advanced stages of regrowth is uncertain but these results suggest (i) a late dependence on root reserves and/or (ii) that the photosynthetic capacity of the first expanded leaves was compromised to a level that limited resource availability for the expansion of subsequent leaves (Fletcher, 2004; Meuriot *et al.*, 2005). The expansion of axillary leaves was relatively more compromised by the low levels of endogenous reserves than the area of primary leaves. The SS treatment had approximately half of the area of axillary leaves than LL treatment (Figure 9). In fact, axillary leaves were extremely important for canopy formation as they comprised 25 to 60% of the final LAI,

mainly during periods of rapid growth (e.g. summer) and in crops with higher levels of endogenous reserves (e.g. LL treatment).

Seasonal differences in canopy expansion

There were also seasonal differences in the area of individual leaves, regardless of treatment. The area of the largest leaf was 60% lower in primary leaves and 90% lower in axillary leaves in spring than summer. Smaller leaves during spring were previously observed in 'Grassland Kaituna' and could be caused by sub-optimal temperatures for growth processes during leaf formation in winter (Brown *et al.*, 2005). The extensibility of cell walls to turgor pressure declines at low temperatures and this limits cell expansion (Pollock, 1990; Tardieu *et al.*, 1999). Alternatively, a lack of assimilates to form new leaves could reduce potential leaf area mainly in the early phases of cell division, when the leaf is heterotrophic (Tardieu *et al.*, 1999).

Developmental processes

Developmental processes of LAI formation (e.g. leaf appearance, branching and shoot initiation) were much less sensitive to defoliation treatments and the level of endogenous reserves than growth processes (e.g. leaf expansion). The phyllochron was conservative at 34°Cd at photoperiods <12.5 h (Figure 5) suggesting that temperature was indeed the main driver of primary leaf appearance during spring/summer (Hodges, 1991). Nevertheless, during autumn-winter (Pp<12.5 h), phyllochron increased in all treatments.

An autumn increase in the phyllochron was previously observed in 'Grassland Kaituna' (Brown *et al.*, 2005) but the physiological mechanisms causing the seasonality of leaf appearance rates remain unclear. A first possibility would be the enhanced activity of specific genes in response to short photoperiods, as assumed in the model for long-day plants proposed by Yan and Wallace (1998). Alternatively, high values of phyllochron in autumn may be caused by limited availability of assimilates to grow shoots (Brown *et al.*, 2005). During autumn dry matter is preferentially partitioned to crowns and roots rather than shoots (Teixeira *et al.*, 2007b) which could restrict the supply of C and N to shoots. Nevertheless, developmental processes seem to be affected by assimilate supply only at extreme levels of stress (Grant and Barthram, 1991) which explains the insensitiveness of the rates of branching (Figure 6). Similarly, maximum shoot population was unaffected by treatments and the rates of shoot appearance and mortality were mainly controlled by temperature and canopy light environment, respectively (Teixeira *et al.*, 2007a). This suggests that the extent by which defoliation treatments reduced the level of endogenous reserves was insufficient to limit basal bud initiation or affect axillary leaf appearance. The exponential increase in branching (i.e. the number of axillary buds) observed for 'Kaituna' (Figure 6) was consistent with the common pattern observed for other legume crops (Ranganathan *et al.*, 2001).

Leaf senescence

The senescence of primary leaves was unaffected by defoliation treatments (Figure 7). Hence the low availability of endogenous reserves, created in the SS treatment, did not

accelerate the remobilization of assimilates from older leaves to expanding ones as could be expected in lucerne crops (Lemaire *et al.*, 1985). There was an acceleration in the rate of senescence after the 6th main-stem node position (Figure 7) when LAI approached 2.0 in 'Grassland Kaituna' (Teixeira, 2006). The decay of shoot population (self-thinning) of 'Kaituna' also resumed at an LAI of 2.0 (Teixeira *et al.*, 2007a) which suggests that competition for light triggered senescence of leaves and shoot death simultaneously. These rates of senescence differ slightly from Brown et al. (2005b) who observed 1.08 senesced leaves/main-stem node occurring after the 9th main-stem node, when canopy was near LAI_{crit}. These differences may be caused by the contrasts in shoot population but, in practical terms, they have a minor impact on yield because the area of senesced leaves in the first 42 days of regrowth does not influence PAR interception.

Physiological and modelling implications

Frequent defoliations reduced shoot growth rates directly through reducing the time available for PAR interception and indirectly by limiting the accumulation of endogenous reserves, which in turn reduced the rate of canopy expansion in the subsequent cycles. Canopy architecture was unaffected by defoliation treatments or the level of endogenous reserves. The implication of a single and conservative k_d value is that, once LAI is known, PAR_i can be produced for crops subjected to different environments and managements. This becomes a sound framework from which to estimate carbon assimilation in lucerne simulation models (Gosse *et al.*, 1984). Developmental processes of canopy formation were less sensitive to environmental and management factors. The

use of a conservative phyllochron of 34°Cd/leaf (Tb =5ºC), as already in the model APSIM-lucerne (Robertson *et al.*, 2001), was able to represent leaf appearance in spring/summer. However, the use of a critical photoperiod (Hodges, 1991) of 12.5 h may improve the accuracy for simulating leaf appearance in autumn/winter when the values of the phyllochron increase. Branching pattern, leaf senescence rates and shoot appearance rates may be accurately derived from cultivar phenology and air temperature, being unaffected by defoliation treatments and the levels of root reserves observed in the current experiment. The expansion of individual primary and axillary leaves was the main component that modulated canopy expansion (i.e. LAER) in response to the seasonal environment and defoliation management. The strong relationship between LAER and the pool of root nitrogen suggests that a better understanding of N balance and N fluxes in lucerne plants may be required to predict the effects of defoliation regimes on lucerne yield. Additional seasonal and treatment yield differences during specific regrowth cycles may be explained by changes in radiation use efficiency (RUE) and the partitioning of DM to roots, which are both important physiological aspects to be further analysed in these lucerne crops.

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Tables

Table 1. Details of the treatments applied to a lucerne crop and respective acronyms for an experiment at Lincoln University, Canterbury, New Zealand from 14 June 2002 to 4 October 2004.

Treatment Acronym	Grazing cycle (days)		Date of the first defoliation in each season (± 4 days)
	Before 4th February	After 4th February	
LL	42	42	1 October
LS	42	28	1 October
SL	28	42	15 September
SS	28	28	15 September

Treatment acronyms are a combination of long (L, 42 days) and short (S, 28 days) regrowth cycles. The grazing duration was on average 4 days for long regrowth cycles (i.e. 38 days of rest) and 3 days for the short regrowth cycle (i.e. 25 days of rest).

Figure captions

Figure 1. Annual accumulated shoot yield as a function of accumulated intercepted PAR ($\sum PAR_i$) of lucerne crops subjected to four contrasting defoliation regimes in the 2002/03 and 2003/04 growth seasons at Lincoln University, Canterbury, New Zealand. Note: Intercept was forced to zero because it was not significant at α=0.05. Shoot yields for

treatments LS and SL in 2002/03 were not collected in the period prior to 4 February 2003. Treatment acronyms are given in Table 1.

Figure 2. Fractional PAR interception as a function of leaf area index of lucerne crops subjected to four contrasting defoliation regimes in the 2003/04 growth season at Lincoln University, Canterbury, New Zealand. Treatment acronyms are given in Table 1.

Figure 3. Seasonal leaf area index of lucerne crops subjected to four contrasting defoliation regimes in the 2002/03 and 2003/04 growth seasons at Lincoln University, Canterbury, New Zealand. Note: Bars represents one standard error of the mean (SEM) pooled among regrowth cycles within each treatment. Numbers indicate the order of regrowth cycles of each growing season. Shoot DM samples were not collected in LS and SL treatments prior to 4 February 2003. The horizontal dotted line represents the critical LAI of 3.6 for reference. Treatment acronyms are given in Table 1.

Figure 4. Leaf area expansion rate (LAER) during the first spring regrowth in relation to the percentages (a, b and c) and total taproot pools (d, e and f) of endogenous reserves from samples harvested in the previous winter period from lucerne crops subjected to four contrasting defoliation regimes in the 2002/03 and 2003/04 growth seasons at Lincoln University, Canterbury, New Zealand. Note: Bars represent one SEM for n=2. Treatment acronyms are given in Table 1. Open squares (Î) refer to LL in the spring of

2002/03 (before other treatments were applied), other treatment/year combinations represented by symbols as in Figure 5.

Figure 5. Relationship between phyllochron and mean photoperiod of lucerne crops subjected to four contrasting defoliation regimes in the 2002/03 and 2003/04 growth seasons at Lincoln University, Canterbury, New Zealand. Note: Arrow indicates Pp of 12.5 h, when phyllochron was 5% greater than asymptote (34°Cd) for LL crops.

Figure 6. Total count of expanded leaves (primary plus axillary) in relation to main-stem leaves (primary) of lucerne crops subjected to four contrasting defoliation regimes at Lincoln University, Canterbury, New Zealand. Note: Dashed line represents x=y. Arrows indicate points of inflection for the start of branching (x=4.0) and acceleration of branching rate (x=8.7). R^2 is pooled for both equations**.**

Figure 7. Number of primary senesced leaves per main-stem node position of lucerne crops subjected to four contrasting defoliation regimes in the 2003/04 growth seasons at Lincoln University, Canterbury, New Zealand. Note: Arrows indicate estimated point of senescence initiation (x=3.6) and bi-linear model breakage (x=6.3) when senescence rate accelerates.

Figure 8. Area of primary leaves at each node position from the base of the main-stem of lucerne crops subjected to a long (42 days, LL) or short (28 days, SS) defoliation interval

in the 2002/03 and 2003/04 growth seasons at Lincoln University, Canterbury, New Zealand. Note: Bars indicate one pooled SEM for each node position. Bell-shaped curves are: (a) LL: $y=927\exp(0.07(x-8.0)^2+0.0019(x-8.0)^3)$; SS: $y=575\exp(0.04(x-7.3)^2-0.0016(x-7.3)^3)$; (b) LL: $y=1293\exp(0.04(x-7.2)^2+0.0039(x-7.2)^3)$; SS: $y=983\exp(0.04(x-6.4)^2-0.0031(x-6.4)^3)$;(c) LL: $y=1850\exp(0.05(x-7.1)2+0.0020(x-7.1)^3)$; SS: $y=1411\exp(0.04(x-6.4)^2-0.004(x-6.4)^3)$. All equations $R^2>0.95$ apart from (b) SS where $R^2=0.85$. Harvest dates and thermal-time accumulation are displayed in each graph.

Figure 9. Area of axillary leaves at each node position from the base of the main-stem of lucerne crops subjected to a long (42 days, LL) or short (28 days, SS) defoliation interval in the 2002/03 and 2003/04 growth seasons at Lincoln University, Canterbury, New Zealand. Note: Bars indicate one pooled SEM for each node position. Fitted models are: (a) LL: $y=361\exp(0.13(x-4.7)^2+0.0019(x-4.7)^3)$; SS: $y=94\exp(0.07(x-4.2)^2-0.0016(x-4.2)^3)$; (b) LL: $y=1320\exp(0.06(x-7.3)^2+0.0039(x-7.3)^3)$; SS: $y=877\exp(0.09(x-6.3)^2-0.0031(x-6.3)^3)$; (c) LL: $y=2468\exp(0.14(x-6.4)^2+0.0020(x-6.4)^3)$; SS: $y=1197\exp(0.12(x-5.9)^2-0.004(x-5.9)^3)$. All equations $R^2>0.97$ apart from (a) SS $R^2=0.62$ and (b) LL $R^2=0.87$. Harvest dates and thermal-time accumulation are displayed in each graph.